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Eicosanoid production by isolated glomeruli of rats with unilateral ureteral obstruction

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Eicosanoid production by isolated glomeruli of rats with unilateral ureteral obstruction. The production of PGE_2 6-keto $PGF_{1\alpha}$ and TxB_2 under basal conditions and after exposure to angiotensin II was examined in vitro in isolated glomeruli from sham-operated control rats and rats with unilateral ureteral obstruction of 24 hour duration, that were or were not pretreated with an inhibitor of the angiotensin I converting enzyme (ACE). Basal prostanoid production was greater in glomeruli from the obstructed kidney (OK) than in glomeruli from the contralateral kidney (CLK) of rats with obstruction or glomeruli from the kidneys of sham-operated rats. Glomeruli obtained from the CLK of rats with unilateral obstruction also produced more PGE_2 and 6-keto $PGF_{1\alpha}$ than glomeruli obtained from kidneys of sham-operated rats. Administration of an ACE inhibitor to rats with unilateral obstruction in vivo returned basal prostanoid production in vitro to levels seen in glomeruli of sham-operated rats. The increase in prostanoid production in response to angiotensin II added in vitro was less in glomeruli from rats with unilateral obstruction than in glomeruli from sham-operated rats. However, the response was restored to that seen in glomeruli of sham-operated rats after blockade of angiotensin II synthesis in vivo in rats with unilateral obstruction. Blockade of angiotensin II synthesis in sham-operated rats did not affect prostanoid synthesis by their glomeruli. The results indicate that endogenous angiotensin II has an important role in the increased synthesis of prostanoids found not only in glomeruli of the OK but also of the CLK of rats with unilateral obstruction and that the in vitro prostanoid production in response to angiotensin II can be restored to that seen in sham-operated rats when the synthesis of angiotensin II is inhibited in vivo.

Available evidence suggests increased release of both vasodilatory prostaglandins, PGE_2 and prostacyclin, and a significant increase in the production of the vasoconstrictor, thromboxane A_2, by the obstructed kidney. Indeed, administration of inhibitors of the cyclooxygenase, in the setting of prior inhibition of the thromboxane synthase, after release of obstruction in rats markedly decreases whole kidney GFR and renal plasma flow [1]. These data suggest that after 24-hours of ureteral obstruction vasodilatory prostaglandins, PGE_2 and prostacyclin, may prevent further decrements in GFR by antagonizing the vasoconstrictive effects of thromboxane A_2.

Presumably this greater production of prostanoids by the obstructed kidney is related in part to increased synthesis of these compounds by glomerular cells. However, Folkert and Schlondorff [2] who studied the production of eicosanoids by glomeruli obtained from rats with unilateral ureteral obstruction of 24-hours or 72 hours duration found no differences in the production of PGE_2, 6-keto $PGF_{1\alpha}$ (the stable metabolite of prostacyclin) and TxB_2 between glomeruli obtained from the obstructed or the contralateral kidney after 24 hours of obstruction. By 72 hours they found that glomeruli from the obstructed kidney produced greater amounts of 6-keto $PGF_{1\alpha}$ and TxB_2 than the glomeruli from the contralateral kidney. On the other hand, PGE_2 synthesis was greater in glomeruli obtained from the contralateral kidney than in glomeruli from the obstructed kidney. In a recent study, we found that isolated glomeruli obtained from the kidneys of rats with bilateral ureteral obstruction of 24 hours duration produced more PGE_2, 6-keto $PGF_{1\alpha}$ and TxB_2 than glomeruli isolated from the kidneys of sham-operated control rats. To determine if differences in prostanoid production by isolated glomeruli exist between rats with unilateral versus bilateral ureteral obstruction we designed the present experiments. In these studies we compared the production of three prostanoids by glomeruli isolated from the kidneys of sham-operated control rats, and from the obstructed and contralateral kidney of rats with unilateral ureteral obstruction. In addition, we examined the effects of inhibition of angiotensin II synthesis in vivo and the effects of addition of angiotensin II in vitro on prostanoid production by isolated glomeruli obtained from sham-operated control rats or from rats with unilateral ureteral obstruction.

Methods

Chemicals and reagents

Enalaprilat, an angiotensin I converting enzyme (ACE) inhibitor, was from of Merck, Sharp & Dohme (Rahway, New Jersey, USA). Angiotensin II, bovine serum albumin (BSA), DNase I (type II), prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2), 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Collagenase type II was purchased from Worthington Biochemical Corp. (Freehold, New Jersey, USA) and 6-keto $PGF_{1\alpha}$ antibody from Cayman Chemical Co. (Ann Arbor, Michigan, USA).

Experimental animal models

Female Sprague-Dawley rats, purchased from Sasco Inc. (Omaha, Nebraska, USA), and weighing 200 to 225 g were divided into two groups, sham-operated control rats (N = 36)

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and rats with unilateral ureteral ligation ($N = 24$). Half of the animals in each group received five intraperitoneal injections of enalaprilat (5 mg/kg) dissolved in saline at 12 hour intervals, starting 48 hours prior to obstruction or sham operation. In the rats receiving enalaprilat, surgery was performed one hour after the last intraperitoneal injection of the drug. Under light ether anesthesia the rats underwent sham operation or unilateral ureteral ligation. The ureters were exposed through a midline abdominal incision and the left ureter was ligated near the bladder with 4-0 silk as described previously [3]. In sham-operated rats, both ureters were visualized through a midline abdominal incision, but the ureter was not ligated. The animals had no access to food and water after surgery. Kidneys were harvested under anesthesia 24 hours after surgery in both groups of rats (see below).

Kidney perfusion and preparation of glomeruli

The kidneys were perfused and the glomeruli isolated using a slight modification of the method of Schreiner et al [4]. After opening the abdominal cavity under pentobarbital anesthesia (5 mg/100 g body wt given intraperitoneally), both kidneys were perfused with 200 to 250 ml of phosphate buffered saline injected above the bifurcation of the aorta. The kidneys were then removed and decapsulated. The cortices were dissected on ice and glomeruli were obtained by pressing slices of renal cortex through three consecutive sieves (mesh size 250, 150 and 75 μm). Glomeruli were suspended in Hanks' balanced salt solution and then washed twice with Hanks' solution by resuspension/centrifugation. The glomeruli were then treated with 60 U/ml collagenase type II and 0.03 mg/ml DNase with shaking (140 cycles/min) for 30 minutes at 37°C to remove Bowman's capsule. After treatment the preparations were washed three times with cold Hanks' solution and then suspended in warm Hanks' solution. Purity of the glomeruli and removal of Bowman's capsule were assessed by light microscopy. Preparations were found to contain more than 90% glomeruli and most were free of Bowman's capsule. No differences in glomerular yield were detected between kidneys of sham-operated rats and either the obstructed or contralateral kidneys of rats with unilateral ureteral obstruction. Glomeruli obtained from each rat were incubated separately. In the case of rats with unilateral obstruction the glomeruli from each kidney were incubated separately.

Incubation of glomeruli

Glomeruli were preincubated in Hanks' solution at 37°C for 30 minutes. Preparations (400 μl) were transferred into plastic centrifuge tubes and incubated without or with increasing concentrations (10^{-10} to 10^{-6} M) of angiotensin II. The hormone was dissolved in 100 μl warm Hanks' solution and the entire 500 μl incubated under continuous agitation (80 cycles/min) at 37°C for 60 minutes. In each experiment, 60 minute incubations were performed also by adding 100 μl of warm Hanks' solution without hormone (basal values). Incubations were terminated by centrifugation (10,000 g for 1 min) at room temperature and the supernatants were stored at −70°C for PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ determinations. The protein content of the pelleted glomeruli was determined by the method of Lowry et al [5] using BSA as a standard. The glomeruli of each rat kidney were incubated separately. Glomeruli were not pooled.

Radioimmunoassay (RIA) of prostanoids

The production of PGE_2, TxB_2 (the stable metabolite of TxA_2) and 6-keto $PGF_{1\alpha}$ (the stable metabolite of prostacyclin) by glomeruli was determined by measuring these prostanoids in the supernatant using specific radioimmunoassays (RIAs). The PGE_2 and TxB_2 antisera were prepared in our laboratory and their cross-reactivities, and details of the RIA have been reported previously [6, 7]. The cross-reactivities of the 6-keto $PGF_{1\alpha}$ antibody (Cayman Chemical, Ann Arbor, Michigan, USA) were 0.05% for PGE_2, 0.07% for TxB_2 and 2.1% for $PGF_{2\alpha}$. When representative assay samples were separated by high-performance liquid chromatography, there was no interfering substance that reacted significantly with 6-keto $PGF_{1\alpha}$ antibody in any sample, excluding the fraction of 6-keto $PGF_{1\alpha}$. All determinations were carried out in duplicate. The RIA was not influenced by the addition of 1 μM angiotensin II to the samples.

Calculations and statistical analysis

Eicosanoid production was corrected for glomeruli protein content and is expressed per mg protein per 60 minute incubation. The increase in eicosanoid production was calculated in absolute amounts (stimulated values after angiotensin II addition minus basal values) or as the ratio of the eicosanoid generation by glomeruli stimulated with increasing concentrations of angiotensin II to the eicosanoid production under basal conditions. Data presented are means ± SD. Statistical analysis was performed by Duncan's multiple range test.

Results

Incubation of glomeruli

Initial studies established the optimal incubation time and amount of glomeruli to be used. We found that eicosanoid synthesis was linear up to approximately 0.3 mg of glomerular protein and was maximal after 60 to 90 minutes of incubation (data not shown). Therefore, the incubation time was 60 minutes and 0.1 to 0.2 mg of glomerular protein were used [8–10].

Basal eicosanoid synthesis by glomeruli from sham-operated rats and rats with unilateral ureteral obstruction

Table 1 shows basal PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ production by glomeruli from kidneys of sham-operated rats or obstructed kidneys and contralateral kidneys of rats with obstruction that received or did not receive enalaprilat prior to surgery. PGE_2 was the most abundant of the three eicosanoids. In glomeruli from sham-operated rats PGE_2 production was 13 and 29 times greater than that of TxB_2 and 6-keto $PGF_{1\alpha}$, respectively.

In glomeruli from the contralateral kidney (CLK) of rats with obstruction not given enalaprilat, PGE_2 production was 15 and 28 times greater than that of TxB_2 and 6-keto $PGF_{1\alpha}$, respectively. In glomeruli from the obstructed kidney (OK) of rats not given enalaprilat, the generation of PGE_2 was 14 and 24 times greater than that of TxB_2 and 6-keto $PGF_{1\alpha}$, respectively. Compared to values obtained in glomeruli from kidneys of sham-operated rats, the basal production of PGE_2 and 6-keto

Table 1. Basal eicosanoid production by glomeruli isolated from sham-operated control rats and rats with unilateral ureteral obstruction that did or did not receive enalaprilat prior to obstruction or sham operation

	Rats not given enalaprilat			Rats given enalaprilat		
	PGE_2	TxB_2	6-keto $PGF_{1\alpha}$	PGE_2	TxB_2	6-keto $PGF_{1\alpha}$
	pg/mg protein/60 min					
SOC	5209 ± 646	398 ± 81	177 ± 51	5281 ± 407	405 ± 29	197 ± 36
CLK	6000 ± 742[a]	393 ± 54	213 ± 33[a]	5367 ± 650	439 ± 62	204 ± 38
OK	10898 ± 954[a,b]	756 ± 130[a,b]	454 ± 86[a,b]	5386 ± 683	425 ± 41	199 ± 15

Glomeruli were prepared from the kidneys of sham-operated rats (SOC) and from the contralateral kidney (CLK) and the obstructed kidney (OK) of rats with unilateral obstruction. Glomeruli were incubated in 500 μl Hanks's balanced salt solution at 37°C for 60 min. The supernatant buffer was assayed for each eicosanoid. Data are corrected for mg protein of glomeruli. Values are means ± SD obtained in eighteen glomerular preparations from 18 sham-operated rats and twelve glomerular preparations from each kidney from 12 rats with unilateral obstruction.

Abbreviations are: PGE_2, prostaglandin E_2, TxB_2, thromboxane B_2; 6-keto $PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$.

[a] $P < 0.01$ compared with each SOC value

[b] $P < 0.01$ compared with each CLK value

$PGF_{1\alpha}$ by glomeruli from the CLK was slightly but significantly greater ($P < 0.01$) by 1.15 and 1.20 times, respectively. The basal production of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ in glomeruli from the OK was significantly greater ($P < 0.01$) by 2.1, 1.9 and 2.6 times, respectively, compared to glomeruli of sham-operated rats. Furthermore, in rats with unilateral obstruction the basal production of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ was significantly greater ($P < 0.01$) by 1.8, 1.9 and 2.1 times, respectively, in the OK than in the CLK. However, the production of TxB_2 in glomeruli from the CLK was comparable and not significantly different from that in glomeruli from kidneys of sham-operated rats. The synthesis of 6-keto $PGF_{1\alpha}$ in glomeruli from the OK compared to that in glomeruli from sham-operated rats was the highest of the three eicosanoids measured and the percentage increase in PGE_2 and TxB_2 generation in glomeruli of the OK versus those of sham-operated rats was almost the same.

The basal production of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ by glomeruli from sham-operated rats given enalaprilat or not given this ACE inhibitor prior to surgery did not differ. Thus, enalaprilat administration in vivo had no significant effect on eicosanoid synthesis by glomeruli from sham-operated rats. By contrast enalaprilat administration in vivo lowered significantly the production of eicosanoids by glomeruli from the obstructed kidney. It also decreased significantly PGE_2 production in the CLK of rats with obstruction. Although 6-keto $PGF_{1\alpha}$ production by the CLK glomeruli of the enalaprilat pretreated rats did not differ significantly from the CLK glomeruli of non-treated rats, this production was not significantly greater than that of glomeruli obtained from sham-operated rats (Table 1). The basal generation of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ by glomeruli from the CLK or OK of rats given enalaprilat did not differ significantly from the values seen in glomeruli obtained from sham-operated rats.

Effects of angiotensin II addition in vitro on eicosanoid synthesis by glomeruli from sham-operated control rats and rats with unilateral ureteral obstruction not given enalaprilat

Figures 1 to 3 show the increase in eicosanoid biosynthesis in response to increasing concentrations of angiotensin II in the incubation medium. In glomeruli from kidneys of sham-operated rats, PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ production was significantly stimulated by 1 nM angiotensin II ($P < 0.01$). PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ synthesis progressively increased up to concentrations of angiotensin II of 100 nM. Maximum responses (expressed as a ratio of basal values) of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ production was 1.7 to 1.8 for each prostanoid. The maximum responses in absolute values are shown in Table 2. In glomeruli from the CLK, the concentrations of angiotensin II which significantly stimulated PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ generation were 10 nM, 10 nM and 1 nM, respectively. The maximum increases in PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ production as a ratio of basal values were about 1.5 at 100 nM angiotensin II. In glomeruli from the OK, the angiotensin II concentration which significantly stimulated PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ was 1 nM, 1 nM and 10 nM, respectively. The maximum increase in PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ synthesis was about 1.3-fold basal values at 100 nM angiotensin II. The increase in eicosanoid generation by glomeruli from the OK expressed as a ratio of basal values was less at 10 or 100 nM angiotensin II concentration ($P < 0.01$), than that seen in glomeruli from kidneys of sham-operated rats and the CLK of rats with obstruction. However, the absolute values for both basal and stimulated prostanoid production in glomeruli from the OK were significantly greater ($P < 0.01$) than those from the CLK or the kidney of sham-operated rats (Table 2). Similarly, the increase in eicosanoid synthesis as a ratio of basal values was significantly less, at least at a 10 nM angiotensin II concentration ($P < 0.01$) in glomeruli from the CLK, when compared to glomeruli from the kidneys of sham-operated rats. In contrast to the results obtained in glomeruli from the OK, the absolute value of TxB_2 for the maximal response observed was significantly lower ($P < 0.01$) in glomeruli from the CLK than in those from the kidneys of sham-operated rats. The absolute maximum-stimulated values of PGE_2 and 6-keto $PGF_{1\alpha}$ in CLK glomeruli were comparable to those seen in glomeruli from sham-operated rats (Table 2).

The absolute increases in eicosanoid production by isolated glomeruli resulting from the in vitro exposure to a maximal stimulatory dose of angiotensin II (100 nM) are shown in Table 3. These values represent the difference in eicosanoid synthesis when the basal values are subtracted from the values measured after incubation of glomeruli in vitro with 100 nM angiotensin II. The data represent the six obstructed and nine sham-operated rats of Figures 1A to 3A.

In rats not given enalaprilat the maximal production of PGE_2,

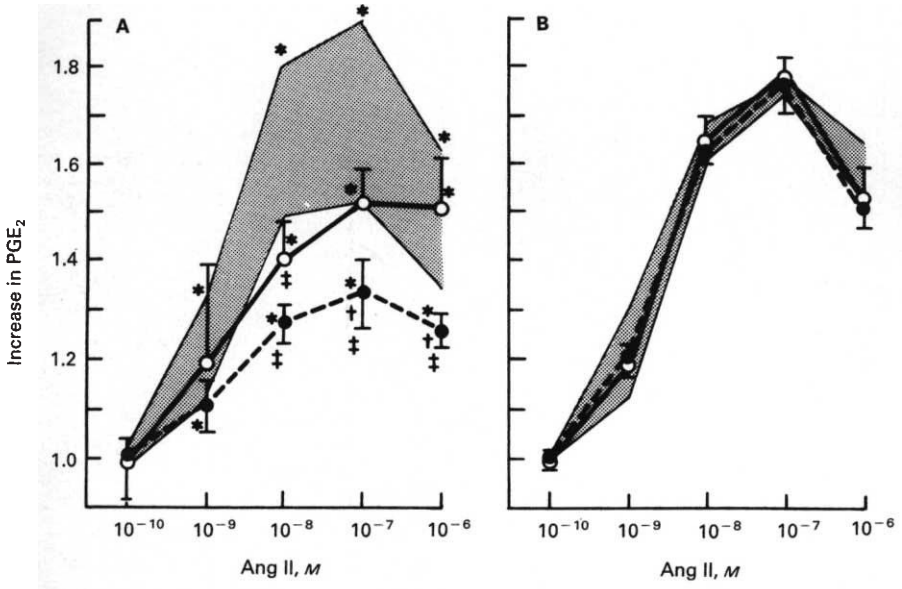

Fig. 1. *Increase in prostaglandin E_2 (PGE_2) production by isolated glomeruli exposed to increasing concentrations of angiotensin II (AngII).* Glomeruli were obtained from kidneys of sham-operated rats (shaded area - mean ± standard deviation) and from the contralateral kidney (○) and the obstructed kidney (●) of rats with unilateral ureteral obstruction that did not **(A)** or did receive **(B)** 5 mg/kg enalaprilat prior to obstruction or sham operation. The increase in PGE_2 was calculated as described in **Methods**. Shaded area represents means ± SD for nine experiments in each A and B. Points represent means ± SD for six experiments in each A and B. (*) $P < 0.01$ vs. basal levels without angiotensin II. (‡) $P < 0.01$ vs. values of sham-operated controls at each AngII concentration. (†) $P < 0.01$ vs. values in contralateral kidneys at each AngII concentration.

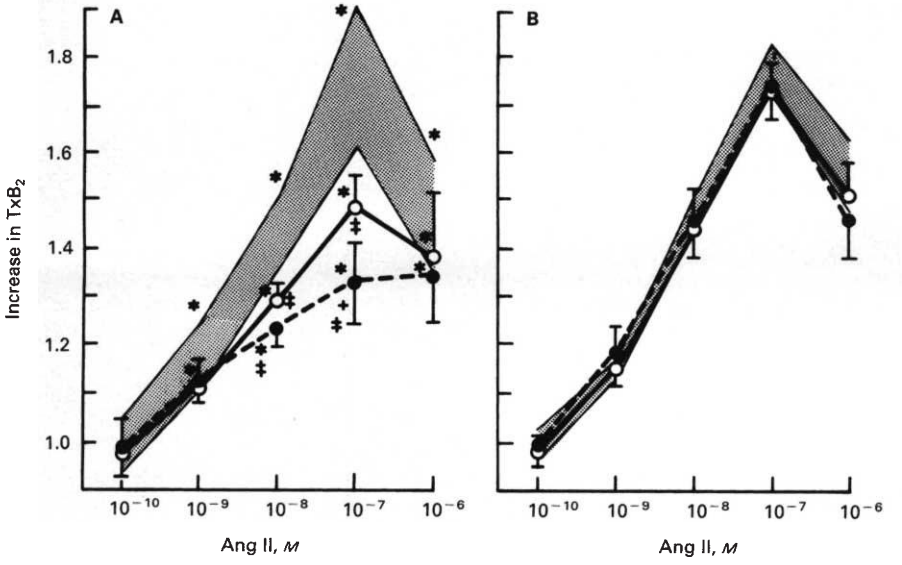

Fig. 2. *Increase in thromboxane B_2 (TxB_2) production by isolated glomeruli exposed to increasing concentrations of angiotensin II (AngII) and prepared from kidneys of sham-operated rats (shaded area) and from the contralateral kidney (○) and obstructed kidney (●) of rats with unilateral ureteral obstruction that did not (A) or did (B) receive 5 mg/kg enalaprilat prior to obstruction or sham operation.* The increase in TxB_2 was calculated as described in **Methods**. Shaded areas represent ± SD for nine experiments. Points represent means ± SD for six experiments. (*) $P < 0.01$ vs. basal levels without angiotensin II. (‡) $P < 0.01$ vs. values of sham-operated controls at each AngII concentration. (†) $P < 0.01$ vs. values in contralateral kidneys at each AngII concentration.

TxB_2 and 6-keto $PGF_{1\alpha}$ with the in vitro addition of angiotensin II was significantly decreased in glomeruli from the contralateral kidney as compared to glomeruli from sham-operated rats. When the absolute differences in maximal eicosanoid production between glomeruli from the obstructed kidney and glomeruli from sham-operated rats were compared only TxB_2 production was significantly decreased, after angiotensin addition in vitro, in glomeruli from the OK from rats not given an ACE inhibitor.

Effects of enalaprilat administration prior to surgery on angiotensin II-stimulated eicosanoid synthesis by glomeruli from sham-operated rats and rats with unilateral ureteral obstruction

As shown in Figures 1B to 3B, increases in PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ production by glomeruli obtained from sham-operated rats or rats with unilateral obstruction given enalaprilat prior to ureteral ligation or sham surgery and exposed to angiotensin II in vitro were comparable. There was no statistical significant difference in the production of the three eicosanoids between the groups. Enalaprilat pretreatment in vivo had no effect on the stimulation of biosynthesis of the three eicosanoids in vitro by angiotensin II in glomeruli isolated from sham-operated rats. Furthermore, the increase in eicosanoid production after addition of angiotensin II to isolated glomeruli obtained from the CLK or the OK of rats given enalaprilat prior to ureteral ligation was comparable to that seen in glomeruli from sham-operated rats.

The maximal values of eicosanoid production when 100 nM angiotensin was added in vitro in glomeruli from sham-operated rats and rats with unilateral obstruction given enalaprilat in vivo are shown in Table 2. There was no statistical significant

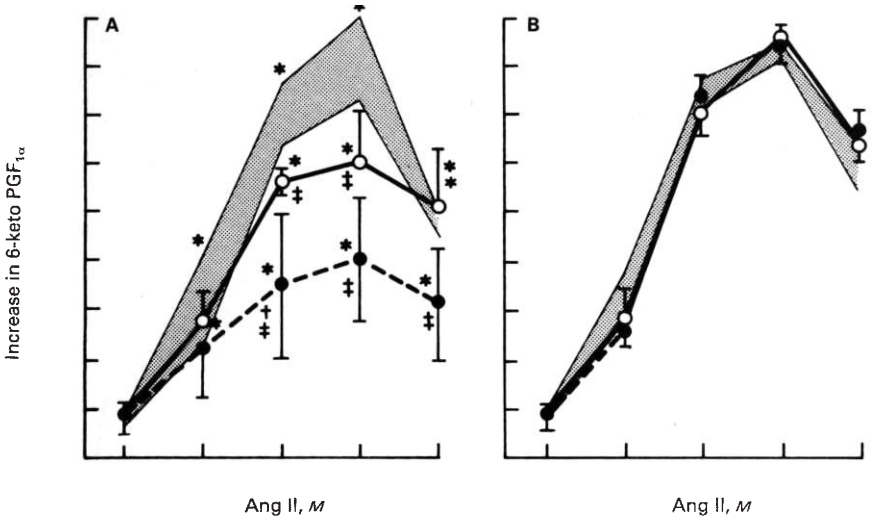

Fig. 3. *Increase in 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) production by isolated glomeruli exposed to increasing concentrations of angiotensin II (AngII) and prepared from the kidneys of sham-operated rats (shaded area) and from the contralateral kidney (○) and the obstructed kidney (●) of rats with unilateral ureteral obstruction that did not (A) or did (B) receive 5 mg/kg enalaprilat prior to obstruction or sham operation.* The increase in 6-keto $PGF_{1\alpha}$ was calculated as described in **Methods**. Shaded areas represent means ± SD for nine experiments. Points represent means ± SD for six experiments. (*) $P < 0.01$ vs. basal levels without angiotensin II. (‡) $P < 0.01$ vs. values of sham-operated controls at each AngII concentration. (†) $P < 0.01$ vs. values in contralateral kidneys at each AngII concentration.

Table 2. Absolute values of maximum eicosanoid production in response to addition of 100 nM angiotensin II to isolated glomeruli from sham-operated control rats and rats with unilateral ureteral obstruction that did or did not receive enalaprilat prior to obstruction or sham operation

	Rats not given enalaprilat			Rats given enalaprilat		
	PGE_2	TxB_2	6-keto $PGF_{1\alpha}$	PGE_2	TxB_2	6-keto $PGF_{1\alpha}$
	pg/mg protein/60 min					
100 nM Angiotensin II						
SOC	9135 ± 1337	711 ± 63	305 ± 51	9221 ± 809	719 ± 42	337 ± 21
CLK	8877 ± 536	584 ± 55[a]	310 ± 17	9688 ± 1402	769 ± 78	346 ± 52
OK	14449 ± 770[a,b]	992 ± 72[a,b]	584 ± 73[a,b]	9260 ± 902	742 ± 90	346 ± 16

Glomeruli were prepared from the kidneys of sham-operated rats (SOC) and from the contralateral kidney (CLK) and the obstructed kidney (OK) of rats with unilateral obstruction. Glomeruli were incubated in 500 μl Hanks's balanced salt solution containing 100 nM angiotensin II at 37°C for 60 min. The supernatant buffer was assayed for each eicosanoid. Data are corrected for mg protein of glomeruli and represent absolute values of maximum eicosanoid production in the presence of 100 nM angiotensin II. Values are means ± SD obtained from nine preparations in nine sham-operated rats and six preparations in 6 rats with unilateral obstruction. Abbreviations are: PGE_2, prostaglandin E_2; TxB_2, thromboxane B_2; 6-keto $PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$.

[a] $P < 0.01$ compared with each SOC value

[b] $P < 0.01$ compared with each CLK value

Table 3. Absolute increase in eicosanoid production in response to addition of 100 nM angiotensin II to isolated glomeruli from sham-operated control rats and rats with unilateral ureteral obstruction that did or did not receive enalaprilat in vivo prior to study

	Rats not given enalaprilat			Rats given enalaprilat		
	PGE_2	TxB_2	6-keto $PGF_{1\alpha}$	PGE_2	TxB_2	6-keto $PGF_{1\alpha}$
	pg/mg protein/60 min					
SOC	3717 ± 782	305 ± 33	128 ± 23	3979 ± 323	318 ± 24	143 ± 8
CLK	2901 ± 428[a]	188 ± 6[b]	102 ± 11[b]	4206 ± 594[c]	322 ± 16[c]	148 ± 20[c]
OK	3574 ± 663	235 ± 49[b]	139 ± 20	3989 ± 284	314 ± 28[c]	147 ± 9

The values presented in this Table are mean ± SD of the differences between basal eicosanoid production and maximal eicosanoid production after addition of 100 nM angiotensin II of Figures 1A to 3A. Glomeruli were prepared from sham-operated control rats (SOC) and from the contralateral kidney (CLK) and obstructed kidney (OK) of rats with unilateral ureteral obstruction. Abbreviations as in Tables 1 or 2.

[a] $P < 0.025$ and [b] $P < 0.01$ compared to each SOC values

[c] $P < 0.01$ compared to no enalaprilat

difference between the values obtained in sham-operated rats and rats with unilateral obstruction with prior ACE inhibition.

No differences in the maximal response to angiotensin II were observed between glomeruli of sham-operated rats that were given or not given the ACE inhibitor in vivo (Table 3). The angiotensin II stimulated increase of all three prostanoids was significantly greater in glomeruli obtained from the contralateral kidney of enalaprilat pretreated rats compared to non-treated rats (Table 3). The angiotensin II-stimulated production of TxB_2 was significantly greater in glomeruli obtained from the OK of rats pretreated with the ACE inhibitor compared to non-treated rats. The absolute increase in PGE_2 and 6-keto $PGF_{1\alpha}$ in

response to addition of angiotensin II in vitro was greater in glomeruli from the OK of enalaprilat pretreated rats compared to non-treated rats, but this did not achieve statistical significance (Table 3).

Discussion

Glomeruli isolated from the obstructed kidney of rats with unilateral ureteral obstruction of 24 hours duration, incubated in vitro, produced significantly greater amounts of PGE_2, prostacyclin (as measured by the release of its stable metabolite, 6-keto $PGF_{1\alpha}$) and TxA_2 (as measured by the release of its stable metabolite, TxB_2) than the glomeruli obtained from the contralateral kidney of the same animals or the kidneys of sham-operated rats. These results resemble those obtained in the obstructed kidney of rabbits perfused in vitro [5, 11, 12]. The basal production rates of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ was between 2 and 26 times greater in glomeruli from the obstructed kidney than in glomeruli from kidneys of sham-operated rats. The basal generation of the three prostanoids in glomeruli from the obstructed kidney was about twofold greater than that observed in glomeruli from the contralateral kidney of the same rats. Isolated glomeruli from the contralateral kidney of rats with unilateral obstruction also generated slightly but significantly greater amounts of PGE_2 and 6-keto $PGF_{1\alpha}$ than glomeruli from kidneys of sham-operated rats. The production of TxB_2 in glomeruli from the contralateral kidney of rats with obstruction was comparable to the value observed in glomeruli from sham-operated rats. These results indicate that unilateral ureteral obstruction of 24 hours duration altered the production of eicosanoids not only in glomeruli isolated from the obstructed kidney but also in glomeruli isolated from the contralateral kidney of the same rats.

Glomeruli from kidneys of sham-operated rats or the obstructed and contralateral kidney of rats with unilateral obstruction produced greater amounts of PGE_2 than of TxB_2 or 6-keto $PGF_{1\alpha}$. The relative order of production of these prostanoids by glomeruli was $PGE_2 > TxB_2 >$ 6-keto $PGF_{1\alpha}$. This pattern resembles that seen in glomeruli from normal rats not subjected to sham-operation [8–10]. Treatment of rats with an inhibitor of the angiotensin I converting enzyme prior to obstruction decreased significantly the production of all three prostanoids by glomeruli from the obstructed kidney and decreased PGE_2 and 6-keto $PGF_{1\alpha}$ by glomeruli from the contralateral kidney. The production of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ by glomeruli from the obstructed kidney as well as PGE_2 and 6-keto $PGF_{1\alpha}$ by glomeruli from the contralateral kidney of rats pretreated with enalaprilat were comparable and not significantly different from the production of these three prostanoids by glomeruli obtained from sham-operated rats. Enalaprilat administration did not affect prostanoid production in glomeruli from sham-operated controls. These results indicate that angiotensin II has a major role in prostanoid production by glomeruli at 24 hours after the onset of unilateral ureteral ligation. Indeed, inhibition of angiotensin II synthesis in vivo resulted in "normalization" of prostanoid production by isolated glomeruli obtained from the obstructed kidney.

Addition of angiotensin II to isolated glomeruli from sham-operated rats increased significantly the production of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ in rats given or not the ACE inhibitor in vivo prior to study. By contrast the absolute increase in prostanoid synthesis in response to angiotensin II was less in glomeruli isolated from the contralateral kidney of rats with unilateral ureteral obstruction not given enalaprilat (Table 3). When rats with unilateral obstruction were pretreated with enalaprilat, the prostanoid production by glomeruli from the CLK in response to angiotensin II was not significantly different to that observed in glomeruli obtained from sham-operated control rats. Thus, glomeruli obtained from the CLK had a slight but significant increase in the basal production of PGE_2, and 6-keto $PGF_{1\alpha}$ and a decreased response in the synthesis of the three prostanoids when these glomeruli were exposed to angiotensin II in vitro. Pretreatment of rats with unilateral obstruction with enalaprilat decreased prostanoid production in glomeruli from the CLK (Table 1) and restored the in vitro response to angiotensin II to levels similar to those seen in glomeruli from sham-operated control rats (Table 3). These findings of an in vitro blunting of the response to angiotensin II may be explained by down regulation of angiotensin II receptors in glomeruli from the CLK of untreated rats.

Of interest in the present study is the finding that glomeruli isolated from the CLK of rats with unilateral obstruction produced more PGE_2 and 6-keto $PGF_{1\alpha}$ than glomeruli obtained from sham-operated controls. This finding may suggest that the generation of PGE_2 and 6-keto $PGF_{1\alpha}$ in glomeruli from the CLK of rats with unilateral obstruction was increased in response to greater levels of circulating angiotensin II in vivo occurring as a consequence of ureteral obstruction. The greater basal production of the three prostanoids by glomeruli isolated from the obstructed kidney as compared to the contralateral kidney may be due to differences in levels of angiotensin II between the two kidneys in vivo. Indeed, intrarenal angiotensin II is very likely higher in the obstructed than in the contralateral kidney of rats with unilateral obstruction. This would also explain the greater inhibition in prostanoid synthesis observed in glomeruli obtained from the obstructed kidney than in glomeruli from the contralateral kidney after inhibition of angiotensin II production in vivo (Table 1).

Another interesting finding of this study is that in response to a maximal dose of angiotensin II the absolute differential production of only TxB_2 was significantly decreased in glomeruli obtained from the obstructed kidney of rats with unilateral ureteral obstruction of 24 hours duration (Table 3). The absolute increase in the production of the vasodilatators PGE_2 and 6-keto $PGF_{1\alpha}$ in response to angiotensin II by glomeruli from the obstructed kidney was not significantly different from the absolute increase seen in glomeruli from sham-operated control kidney glomeruli (Table 3). Prior treatment with the ACE inhibitor in vivo increased the TxB_2 production by glomeruli from the obstructed kidney in vitro to levels similar to those seen in glomeruli obtained from sham-operated control rats.

It has been shown previously that the activities of both phospholipase and cyclooxygenase are increased in the kidney with ureteral obstruction [13, 14]. The mechanisms by which ureteral obstruction increases the activity of these enzymes is not known. Angiotensin II release during obstruction may be one of the mechanisms responsible for the increased activity of either or both phospholipase A_2 and cyclooxygenase. Angiotensin II may stimulate the production of prostanoids by

increasing the availability of arachidonic acid as a consequence of an effect of the hormone on the activity of phospholipase [6, 15]. Consequently, it is possible that the differences in prostanoid production between the CLK and OK elicited in vitro, is related to differences in the activities of the phospholipase in the two kidneys as a consequence of their exposure to different levels of angiotensin II in vivo. The contralateral kidney being exposed to increased levels of circulating angiotensin II, whereas the obstructed kidney would be influenced by both circulating and local angiotensin II.

In addition to the aforementioned increased in phospholipase and cyclooxygenase activities in obstruction, there is a decrease in thromboxane synthase activity in both the contralateral and obstructed kidney of rats one day after unilateral ureteral ligation, although there is a dramatic increase in activity thereafter [16]. This could explain the present observation that less TxB_2 production was observed in glomeruli from the CLK. A possible factor contributing to the decreased TxB_2 production in glomeruli from the obstructed kidney could be the decrease in the number of resident macrophages seen in glomeruli from the OK after 24 hours of obstruction [3]. The decreased macrophage content could disrupt the interaction with glomerular cells [17], thus altering the profile of eicosanoid products.

Folkert and Schlondorff [2] studied the production of prostaglandins by glomeruli from rats with unilateral ureteral obstruction of 24 and 72 hours duration. They found that production of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ was not different between glomeruli obtained from the obstructed and the contralateral kidney after 24 hours of unilateral obstruction. By 72 hours they found that glomeruli from the obstructed kidney produced more TxB_2 and 6-keto $PGF_{1\alpha}$ than glomeruli from the contralateral kidney. On the other hand, PGE_2 synthesis was higher in glomeruli obtained from the contralateral, untouched, kidney than in glomeruli from the obstructed kidney. These results are somewhat different from ours. The differences may relate to the experimental conditions used: female rats in our study versus male rats in the study of Folkert and Schlondorff [2], glomeruli isolated from perfused kidneys in this study versus glomeruli isolated from non-perfused kidneys, glomeruli without Bowman's capsule versus glomeruli with Bowman's capsule, and 60 minutes of incubation in our study versus 10 minutes of superfusion in the study of Folkert and Schlondorff [2].

Of importance is also the fact that we studied sham-operated control rats. We found that in rats with unilateral obstruction the contralateral kidney "behaved" differently than the kidney of sham-operated rats in terms of eicosanoid production. Compared to the results obtained in glomeruli of sham-operated control rats, glomeruli from the contralateral kidney of rats with unilateral obstruction had 1) an increased production of PGE_2 and 6-keto $PGF_{1\alpha}$, and 2) a decreased response in the production of the three eicosanoids in response to angiotensin II added in vitro. McNamara et al have also indicated that the non-obstructed contralateral kidney of rats with unilateral ureteral obstruction may not be suitable as a control for the obstructed kidney because of the altered metabolism of microsomal PGH_2 in the contralateral kidney 24 hours after the onset of obstruction when compared to that observed in normal control kidneys [16].

In summary, glomeruli isolated from the OK of rats with unilateral obstruction of 24 hours duration produced greater amounts of PGE_2, TxB_2 and 6-keto $PGF_{1\alpha}$ than isolated glomeruli from the CLK or the kidneys of sham-operated rats. Similarly, the production of PGE_2 and 6-keto $PGF_{1\alpha}$ was greater in glomeruli from the CLK than in glomeruli from sham-operated control rats. This increased generation of prostanoids by isolated glomeruli from rats with unilateral obstruction is reversed by blocking angiotensin II generation in vivo. This same maneuver, however, did not affect prostanoid production in isolated glomeruli from sham-operated control rats. It is concluded that in rats with unilateral ureteral obstruction of 24 hours duration endogenous angiotensin II is the major mechanism responsible for the increased synthesis of prostanoids in glomerular cells of both the contralateral and the obstructed kidney.

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